TITLE OF THE INVENTION

Glucose sensor

BACKGROUND OF THE INVENTION

The present invention relates to a glucose sensor which facilitates rapid and simplified quantitative analysis of a specific component contained in a sample with high accuracy. More specifically, the present invention relates to a method for stabilizing glucose dehydrogenase whose coenzyme is pyrrolo-quinoline quinone and a glucose dehydrogenase composition obtained by the stabilizing method.

Conventionally, a variety of biosensor have been proposed as a system facilitating simple quantitation of a specific component contained in a sample solution without requiring dilution or agitation of a sample solution. The following is a known example of such biosensor (Japanese Laid-Open Patent Publication No. Hei 2-062952).

The biosensor disclosed in this prior art is produced by the steps of forming an electrode system including a working electrode, a counter electrode and a reference electrode on an electrically insulating base plate using a known screen printing method or the like and subsequently forming immediately on this electrode system an enzyme reaction layer containing a hydrophilic polymer, an oxidoreductase, and an electron acceptor.

Upon a dropwise addition of a sample solution containing a substrate over the enzyme reaction layer of

2

the biosensor thus produced, the enzyme reaction layer dissolves in the sample solution and the substrate in the sample solution is oxidized by the enzyme. At that time, the electron acceptor is reduced. After enzyme reaction is completed, the reduced electron acceptor is electrochemically reoxidized. The concentration of the substrate in the sample solution can be determined based on the oxidation current produced by the reoxidation reaction.

In principle, the biosensor as described above permits measurements of various materials if a suitable enzyme corresponding to the substrate of an analyte is selected.

For example, if glucose oxidase is the selected enzyme, then a glucose sensor for measuring a glucose concentration in the sample solution can be obtained.

The biosensor of the above structure normally accommodates the enzyme in the dried state. However, the enzyme is susceptible to degeneration when exposed to water in air for a long time, because it is essentially composed of protein which is readily degraded. In the extreme, the enzyme is exposed to a risk of losing the enzyme activity.

Therefore, long-term preservation of sensors after their production may result in a loss of activity of the enzyme and a depletion of necessary enzyme for reacting with the substrate. This may lead to a noncommensurable sensor response current to the substrate

concentration.

In general, introduction of a sample solution containing a 0% substrate can produce some degree of sensor response current (hereinafter referred to as "blank value"). One cause of such blank value may be induction of electrode reaction due to an accumulation of ions contained in the sample solution dissolving the reaction layer on the surface of the electrode system formed on the base plate. A large blank value can serve as a factor for impairing the correlation between response current and substrate concentration, rendering it impossible to make precise quantitative analysis of the substrate.

Therefore, securing an environment where the enzyme can retain the enzyme activity for a long term in the vicinity of the enzyme is key to the provision of a biosensor demonstrating excellent stability against preservation and producing a low blank value. It is also important to secure an environment, which produces a minimal and negligible blank value, around the surface of the electrode system on the base plate. It is also necessary to realize smooth transfer of both electron and substrate during enzyme reaction so as to enhance sensor response.

One conventional countermeasure for solving the above-mentioned problems is an inclusion of an additive such as phosphoric acid in the reaction layer.

In order to produce a high performance glucose sensor, on the other hand, glucose dehydrogenase whose

coenzyme is pyrrolo-quinoline quinone (hereinafter abbreviated to "PQQ-GDH") has conventionally been used as the enzyme. If PQQ-GDH is included as the enzyme, the resultant glucose sensor inherently has a characteristic feature of complete freedom from any adverse influence of dissolved oxygen in blood or the like on the enzyme reaction, because oxygen plays no role in the catalytic action of PQQ-GDH. Therefore, measurement values obtained from such glucose sensor are also free of variations due to oxygen partial pressure in the sample solution. This means that a high performance sensor will result from the use of PQQ-GDH as the enzyme.

However, the use of PQQ-GDH as the enzyme has a drawback that even inclusion of an additive such as phosphoric acid as exemplified before in the reaction layer can not help the resultant biosensor to lower the blank value sufficiently and to demonstrate sufficiently high stability against preservation.

BRIEF SUMMARY OF THE INVENTION

In view of the above-mentioned problems, a primary object of the present invention is to provide a high performance glucose sensor demonstrating high stability against preservation and producing a low blank value. Other objects of the present invention are to provide a method for stabilizing PQQ-GDH and a glucose dehydrogenase composition obtained by the stabilizing method.

5

The glucose sensor in accordance with the present invention comprises an electrically insulating base plate, an electrode system including at least a working electrode and a counter electrode formed on the base plate, and a reaction layer which is formed in contact with or in the vicinity of the electrode system and contains at least PQQ-GDH, wherein the reaction layer further contains at least one additive selected from the group consisting of phthalic acid, a phthalate, maleic acid, a maleate, succinic acid, a succinate, triethanol amine, a triethanol amine salt, citric acid, a citrate, dimethyl glutaric acid, 2-(N-morpholino)ethane sulfonic acid, a 2-(N-morpholino)ethane sulfonate, tris(hydroxymethyl)glycine, a tris(hydroxymethyl)glycine salt, tris(hydroxymethyl)aminomethane, a tris(hydroxymethyl)aminomethane salt, imidazole, and colicin.

Sub F1

In a preferred mode of the present invention, the enzyme is coated with the additive.

The present invention also relates to a method for stabilizing glucose dehydrogenase for use in glucose sensors, wherein at least one additive is added to PQQ-GDH, the additive being selected from the group consisting of phthalic acid, a phthalate, maleic acid, a maleate, succinic acid, a succinate, triethanol amine, a triethanol amine salt, citric acid, a citrate, dimethyl glutaric acid, 2-(N-morpholino)ethane sulfonic acid, a 2-(N-morpholino)ethane sulfonate, tris(hydroxymethyl)glycine, a

Sub F2

6

tris(hydroxymethyl)glycine salt, tris(hydroxymethyl)aminomethane, a tris(hydroxymethyl)aminomethane salt, imidazole, and colicin.

Sub F27 Cont'd

The present invention further relates to a glucose dehydrogenase composition for use in glucose sensors, the composition containing PQQ-GDH and at least one additive selected from the group consisting of phthalic acid, a phthalate, maleic acid, a maleate, succinic acid, a succinate, triethanol amine, a triethanol amine salt, citric acid, a citrate, dimethyl glutaric acid, 2-(N-morpholino)ethane sulfonic acid, a 2-(N-morpholino)ethane sulfonate, tris(hydroxymethyl)glycine, a tris(hydroxymethyl)glycine salt, tris(hydroxymethyl)aminomethane, a tris(hydroxymethyl)aminomethane salt, imidazole, and colicin.

Sub F3

JN B17

While the novel features of the invention are set forth particularly in the appended claims, the invention, both as to organization and content, will be better understood and appreciated, along with other objects and features thereof, from the following detailed description taken in conjunction with the drawings.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

FIG. 1 is a schematic plan view illustrating a glucose sensor in accordance with one example of the present invention from which the reaction layer has been

7

omitted.

FIG. 2 is a longitudinal cross-sectional view illustrating the vital parts of the glucose sensor shown in FIG. 1.

FIG. 3 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 1 of the present invention and those from a glucose sensor in accordance with Comparative Example 1.

FIG. 4 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 3 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 5 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 4 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 6 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 5 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 7 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 6 of the present invention and those from the glucose sensor in accordance with Comparative Example 1 with

Comparative Example 1.

FIG. 8 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 7 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 9 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 8 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 10 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 9 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

FIG. 11 is a graph illustrating response characteristics obtained from a glucose sensor in accordance with Example 10 of the present invention and those from the glucose sensor in accordance with Comparative Example 1.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the glucose sensor in accordance with the present invention further contains an additive such as phthalic acid in the reaction layer containing PQQ-GDH as the enzyme.

When the reaction layer is formed by dropping

and drying an aqueous mixed solution of PQQ-GDH with the additive such as hydrogen potassium phthalate, for example, the surface of the enzyme PQQ-GDH is coated with the additive hydrogen potassium phthalate. Such coating protects the enzyme from any change in the environmental conditions such as temperature, humidity, electric charge. As a result, the enzyme can retain stable enzyme activity for a long term.

Furthermore, upon introduction of a sample solution into the sensor, the additive is dissolved in the sample solution and ionized. The ionized additive in turn influences preexisting ions in the sample solution and those produced upon dissolution of the reaction layer in the sample solution. As a result, those ions can not stay on the surface of the electrode system on the base plate, which contributes to minimizing the blank value.

The presence of such additive in the reaction layer is also technically advantageous in that it helps dissolution of the reaction layer in water, which facilitates immediate dissolution of the reaction layer in the sample solution upon addition of the sample solution to the reaction layer, thereby enabling smooth progress of both enzyme reaction and electrode reaction.

Sub F4

The additive from which the above-mentioned effects can be expected may be exemplified as phthalic acid, a phthalate such as potassium hydrogen phthalate, maleic acid, a maleate such as sodium maleate, succinic acid, a succinate such as sodium succinate, triethanol

10

Sub F4 Cont'd

~~amine, a triethanol amine salt such as triethanol amine hydrochloride, citric acid, a citrate such as monopotassium citrate, calcium citrate, tripotassium citrate, trisodium citrate, trilithium citrate, diammonium hydrogen citrate, disodium hydrogen citrate, sodium citrate, diammonium citrate, potassium dihydrogen citrate, sodium dihydrogen citrate, disodium citrate or magnesium citrate, dimethyl glutaric acid, 2-(N-morpholino)ethane sulfonic acid, a 2-(N-morpholino)ethane sulfonate, tris(hydroxymethyl)glycine, a tris(hydroxymethyl)glycine salt, tris(hydroxymethyl)aminomethane, a tris(hydroxymethyl)aminomethane salt such as tris(hydroxymethyl)aminomethane hydrochloride, imidazole, and colicin.~~

Particularly, the use of potassium hydrogen phthalate as the additive produces a glucose sensor exhibiting excellent stability against preservation and superb response characteristics with a very low blank value.

All of the above exemplified additives are compounds which can serve as buffers. At use, they can be adjusted to a desired pH using an acid such as hydrochloric acid, acetic acid or an alkali such as sodium hydroxide, potassium hydroxide if occasion demands. A preferred pH range is 5.0 to 8.5. Those additives may be dissolved in other appropriate buffer solutions at use.

The ratio of the additive may be in a range of 5 to 80 μM per 250 to 10,000 U (= units)/ml of PQQ-GDH.

11

From the aspects of stability and blank value, namely, due to the reason that excess content of the additive decreases specific activity of the enzyme, a preferred ratio of the additive should be in a range of 10 to 50 μM.

According to the present invention, the reaction layer may further contain such electron acceptor that is reduced by enzyme reaction. Applicable electron acceptor for this purpose may be exemplified as ferricyanide ion, p-benzoquinone and a derivative thereof, phenazine methosulphate, methylene blue, ferrocene and a derivative thereof, and the like.

According to the present invention, the reaction layer may further include a hydrophilic polymer. The presence of a hydrophilic polymer in the reaction layer prevents separation or dissection of the reaction layer from the surface of the electrode system. The hydrophilic polymer also has a preventive effect against crack development on the surface of the reaction layer, thereby enhancing the reliability of the resultant biosensor.

Preferred examples of hydrophilic polymer for this purpose include carboxymethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, methyl cellulose, ethyl cellulose, ethylhydroxyethyl cellulose, carboxymethylethyl cellulose, polyvinyl pyrrolidone, polyvinyl alcohol, polyamine such as polylysine, polystyrene sulfonate, gelatin and a derivative thereof, a polymer of acrylic acid and its acrylate, a polymer of methacrylic acid and a methacrylate, starch and a

derivative thereof, a polymer of maleic anhydride and a maleate, agarose gel and a derivative thereof.

The reaction layer may be located at various sites in the biosensor in addition to the location on the electrode system formed on the electrically insulating base plate unless the effects of the present invention would be lost. For example, the reaction layer may be located anywhere in the biosensor apart from the electrode system on the base plate. The biosensor in accordance with the present invention further comprises a cover member. The cover member is combined with the base plate to form, between the cover member and the base plate, a sample solution supply pathway for supplying a sample solution to the electrode system. The reaction layer may be located on an exposed side of the cover member to the sample solution supply pathway.

As to the measurement of oxidation current, there are two methods: one is a two-electrode system comprising only a working electrode and a counter electrode and the other is a three-electrode system further comprising a reference electrode in addition to the two electrodes. The latter facilitates more precise and accurate measurement.

As mentioned before, the present invention also relates to a method for stabilizing glucose dehydrogenase for use in glucose sensors by adding one of the above-exemplified additives to PQQ-GDH. The present invention does not limit the method of addition to particular one

and any method can be applied unless it damages the effects of the present invention.

The present invention is also directed to a glucose dehydrogenase composition for use in glucose sensors that is composed of PQQ-GDH plus the additive.

The stabilized glucose dehydrogenase composition in accordance with the present invention may further contain other stabilizer to the extent not impairing the effects of the present invention, in addition to the above-mentioned additive.

Applicable examples of stabilizer for this purpose include a metal salt, a protein, an amino acid, a sugar, an organic acid, a surfactant and so on.

The metal salt may be exemplified as a halogenide or a halide of calcium, strontium or manganese, their sulfate or nitrate.

Preferred protein is one which does not have any adverse effect on the enzyme activity. Examples of such protein are bovine serum albumin (BSA), egg albumin and gelatin.

Applicable amino acid may be exemplified as glycylglycine and polylysine, in addition to general amino acids such as lysine, histidine and glutamic acid. Above all, a highly water-soluble amino acid is preferable.

As the sugar, any sugar may be used regardless of the species and may be exemplified as monosaccharide, disaccharide, oligosaccharide and polysaccharide. Their derivatives may also be applicable. More specific

examples are glucose, fructose, galactose, mannose, xylose, sucrose, lactose, maltose, trehalose, malt triose, maltosyl cyclodextrin, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, dextrin, amylose, glycogen, starch, inulin, glucosamine, inositol, mannitol, sorbitol, ribitol and deoxyglucose.

Examples of organic acid include α-ketoglutaric acid, malic acid, fumaric acid, gluconic acid, cholic acid, and deoxycholic acid.

Preferable surfactant is non-ionic one.

Among others, boric acid, borax, potassium chloride, sodium chloride, ammonium sulfate, glycerol, Ficoll, EDTA (ethylenediaminetetraacetic acid), EGTA, DTT (dithiothreitol), DTE (dithioerythritol), GSH (glutathione) or 2-mercaptoethanol may also be applicable.

A preferable amount range of those stabilizers is 0.0001 to 1.0 part by weight per 1.0 part by weight of glucose dehydrogenase.

The glucose dehydrogenase composition of PQQ-GDH added with, if necessary, the above-mentioned stabilizer in addition to the additive in accordance with the present invention has low cost and can retain its activity without adverse influence on the intrinsic property of the enzyme.

The coenzyme pyrrolo-quinoline quinone as adopted by the present invention may be derived from any source.

Now, the measurement method of the activity of PQQ-GDH of the present invention will be described.

The reagent used is a mixed reagent solution of 50 mM PIPES buffer solution, 0.2 mM PMS (pH 6.5), 0.2 mM NTB, 30.6 mM glucose and 0.19% Triton X-100.

After heating 3 ml of the mixed reagent solution at 37 °C for about 5 min, a PQQ-GDH solution was added to the heated mixed reagent solution at 0.1 ml and the resultant solution was gently stirred.

Subsequently, the solution thus obtained was measured for its absorbance using a spectrophotometer at a controlled temperature of 37 °C and the absorbance was recorded for 5 minutes. Water was used as control. Then, changes in absorbance per min was calculated from a linear portion on the recorded spectral data. Double blind test was made by measuring absorbance of the mixed reagent solution added with mere distilled water in place of PQQ-GDH solution. The amount of enzyme which produces diformazan at 1/2 μM per min as measured by the above method was defined as one unit.

In the following, the present invention will be described more specifically by way of concrete examples. However, the present invention is not limited only to those examples.

Examples

FIG. 1 is a schematic plan view of a biosensor in accordance with one example of the present invention with an omission of the reaction layer.

As shown in the figure, a silver paste is

printed on an electrically insulating base plate 1 made of polyethylene terephthalate using the known screen printing method in order to form leads 2 and 3. Then, a conductive carbon paste containing a resin binder is printed on the base plate 1 to form a working electrode 4. The working electrode 4 is in contact with the lead 2. The base plate 1 is further formed thereon with an insulating layer 6 by printing an insulating paste. The insulating layer 6 surrounds the periphery of the working electrode 4 to hold the exposed area of the working electrode 4 constant. Then, a ring-like counter electrode 5 is formed on the base plate 1 by printing the same conductive carbon paste containing a resin binder as above such that the paste has a contact with the lead 3.

FIG. 2 is a schematic longitudinal cross-sectional view of the biosensor of FIG. 1. On the base plate 1 as shown in FIG. 1, a hydrophilic polymer layer 7 of carboxymethyl cellulose is formed, on which a reaction layer 8 containing PQQ-GDH plus the additive is further formed.

Example 1

A 0.5 wt% aqueous sodium salt solution of a hydrophilic polymer, carboxymethyl cellulose (hereinafter abbreviated to "CMC"), was dropped on the electrode system on the base plate 1 in FIG. 1 and dried for 10 min in a warm air drier at 50 °C to form the CMC layer 7. Then, a mixed solution dissolving 5,000 U PQQ-GDH, 20 μM potassium

hydrogen phthalate and 50 μM potassium ferricyanide in 1 ml water was dropped on the CMC layer 7 and dried to form the reaction layer 8. In this way, the glucose sensor of Example 1 was produced.

Then, aqueous solutions containing various concentrations of glucose were prepared as sample solutions. Each of the sample solutions thus prepared was dropped on the reaction layer 8. Upon supply of the glucose sample solution to the reaction layer, glucose contained in the sample solution was oxidized by PQQ-GDH present in the reaction layer 8. At that time, potassium ferricyanide in the reaction layer was reduced to potassium ferrocyanide.

One minute after dropping the sample solution, a voltage of +0.5 V was applied onto the working electrode 4 using the counter electrode 5 as reference in order to reoxidize potassium ferrocyanide. Five seconds after voltage application, current flowing across the working and the counter electrodes 4 and 5 was measured.

Current values were obtained from all of the sample solutions containing various concentrations of glucose in the same manner as noted above. Finally, a graph showing response characteristics of the glucose sensor to the sample solutions was prepared by plotting the glucose concentration on the X axis and the current value on the Y axis. The results are shown in FIG. 3.

An identical biosensor was produced in the same manner as described above and preserved at room

temperature for 6 months in order to prepare a graph showing its response characteristics after 6-month preservation. The results are also given in FIG. 3.

As evident from FIG. 3, the biosensors of Example 1 produce an extremely low blank value. It is also noted that there is a certain correlation between the glucose concentration and the current value with sharp linearity.

The sensor response characteristics 6 months after production as shown by "Ex.1-2" were almost unchanged compared to those immediately after production as shown by "Ex.1-1". This indicates that the biosensors of Example 1 can well stand stable against preservation.

Comparative Example 1

Another glucose sensor was produced in the same manner as in Example 1, except for the use of potassium phosphate in place of potassium hydrogen phthalate. Then, a graph of sensor response characteristics immediately after production as shown by "Com.Ex.1-1" and after 6 month-preservation as shown by "Com.Ex.1-2" was prepared in the same manner as in Example 1. The results are shown in FIG. 3.

As apparent from FIG. 3, the glucose sensor of Comparative Example 1 produces a high blank value. The response current value of the sensor is higher than the real current value reflecting the glucose concentration when the glucose concentration in the sample solution is

lower than 110 mg/dl. When the glucose concentration in the sample solution is higher than 110 mg/dl, the response current value is lower in the glucose sensor of Comparative Example 1 than that of Example 1. The linear correlation between the glucose concentration and the current value immediately after production is also lower.

The linear correlation further decreased after 6-month preservation compared to that immediately after production. As such, the glucose sensor of Comparative Example 1 had poor preservation characteristics.

Comparative Example 2

Another glucose sensor was produced in the same manner as in Example 1, except for omission of potassium hydrogen phthalate. Then, a graph of response characteristics of the sensor immediately after production and after 6-month preservation was prepared in the same manner as in Example 1.

The results showed that the sensor of Comparative Example 2 produced a surprisingly high blank value and low increases in the current value in response to increases in the substrate concentration. The enzyme lost its enzyme activity after 6-month preservation of the sensor and there was almost no change in the current value in response to increased substrate concentrations.

Example 2

In this example, a glucose sensor was produced

20

in the same manner as in Example 1, except for omission of the CMC layer 7 on the electrode system. Then, a graph of response characteristics of the sensor immediately after production and after 6-month preservation was prepared in the same manner as in Example 1.

The results showed a certain correlation between the glucose concentration and the current value with satisfactory linearity. The sensor was low in blank value. Moreover, the sensor response characteristics 6 months after production were almost unchanged compared to those immediately after production. This indicates satisfactory stability of the sensor of Example 2 against preservation.

Examples 3 to 10

In these examples, glucose sensors were produced in the same manner as in Example 1 by using maleic acid (Example 3), succinic acid (Example 4), triethanol amine hydrochloride (Example 5), sodium dihydrogen citrate (Example 6), dimethyl glutaric acid (Example 7), 2-(N-morpholino)ethane sulfonic acid (Example 8), tris(hydroxyethyl)glycine (Example 9) or tris(hydroxymethyl)aminomethane (Example 10) in place of potassium hydrogen phthalate. Then, graphs of response characteristics of the sensors immediately after production as shown by "Ex.3-1 to Ex.10-1" and after 6-month preservation as shown by "Ex.3-2 to Ex.10-2" were prepared in the same manner as in Example 1. The results

obtained from the sensors are shown in FIG. 4 to FIG. 11.

FIG. 4 to FIG. 11 indicate very low blank values and a certain correlation between the glucose concentration and the current value. The sensors were highly responsive, demonstrating satisfactory linearity. Moreover, the sensor response characteristics 6 months after production were almost unchanged compared to those immediately after production. This indicates satisfactory stability of the sensors of Examples 3 to 10 against preservation.

Examples 11 to 18

In these examples, glucose sensors were produced in the same manner as in Examples 3 to 10, except for omission of the CMC layer 7 on the electrode system. Then, graphs of response characteristics of the sensors immediately after production and after 6-month preservation were prepared similarly.

The results indicate a certain correlation between the glucose concentration and the current value with satisfactory linearity. The blank value was also very low in all the sensors. Moreover, the sensor response characteristics 6 months after production were almost unchanged compared to those immediately after production. This indicates satisfactory stability of the sensors of Examples 11 to 18 against preservation.

Example 19

In this example, 10 U/ml of the PQQ-GDH in accordance with the present invention was dissolved in various additives (20 mM) each containing 1 mM calcium chloride (which will be called "buffer solutions") and stored at 37 °C for 3 days to examine the residual enzyme activity (more specifically, ratio of remaining enzyme activity of the PQQ-GDH to that immediately after being dissolved in either buffer solution). Calcium chloride was omitted when the buffer was potassium phosphate buffer solution.

Table 1 lists the residual enzyme activity of PQQ-GDH dissolved in the various buffer solutions.

Table 1

Buffer solution	pH	Residual activity (%)
Potassium hydrogen phthalate	6.0	100
Maleic acid	6.5	100
Succinic acid	6.0	100
Triethanol amine	7.0	100
Sodium dihydrogen citrate	6.5	100
Dimethyl glutaric acid	6.5	100
Tricine	7.5	95.4
Imidazole	7.5	100
Colicin	6.5	96.1
Tris hydrochloride	7.5	63.4
Potassium phosphate	6.5	44.3

T,040

Sub F5

Table 1 indicates that all the additives in accordance with the present invention produce better stability than the conventional potassium phosphate buffer solution and tris hydrochloride buffer solution which are widely applied additives.

Example 20

In this example, the PQQ-GDH in accordance with

24

the present invention was dissolved in a variety of additives containing 1 mM calcium chloride in addition to BSA (which will be called buffer solutions). The mixing ratio of BSA was 0.3 part by weight to 1.0 part by weight of PQQ-GDH. The solution thus prepared was lyophilized (=freeze-dried) and stored at 37 °C for 1 week to examine the residual enzyme activity (more specifically, ratio of remaining enzyme activity of the PQQ-GDH to that immediately after being dissolved in either buffer solution). The results are shown in Table 2.

Table 2

Buffer solution	pH	Residual activity (%)
Tris hydrochloride	7.5	22.1
Potassium phosphate	6.5	66.2
Potassium hydrogen phthalate	6.0	83.2
Maleic acid	6.5	72.1
Succinic acid	6.0	80.5

Table 2 indicates that all the additives in accordance with the present invention are superior to the widely used conventional potassium phosphate buffer solution and tris hydrochloride buffer solution in securing stable activity of the enzyme composition prepared by a freeze-drying method.

25

As discussed above, the present invention can provide a high performance glucose sensor which well stands long-term preservation and has a low blank value. The present invention can also provide a glucose dehydrogenase composition bound with pyrrolo-quinoline quinone as coenzyme for use in glucose sensors, the composition being more stable than the conventional glucose dehydrogenase composition.

Although the present invention has been described in terms of the presently preferred embodiments, it is to be understood that such disclosure is not to be interpreted as limiting. Various alterations and modifications will no doubt become apparent to those skilled in the art to which the present invention pertains, after having read the above disclosure. Accordingly, it is intended that the appended claims be interpreted as covering all alterations and modifications as fall within the true spirit and scope of the invention.